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Susceptibilities of *Mycoplasma hominis, M. pneumoniae*, and *Ureaplasma urealyticum* to GAR-936, Dalfopristin, Dirithromycin, Evernimicin, Gatifloxacin, Linezolid, Moxifloxacin, Quinupristin-Dalfopristin, and Telithromycin Compared to Their Susceptibilities to Reference Macrolides, Tetracyclines, and Quinolones

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The susceptibilities of *Mycoplasma hominis*, *Mycoplasma pneumoniae*, and *Ureaplasma urealyticum* to eight new antimicrobial agents were determined by agar dilution. *M. pneumoniae* was susceptible to the new glycylcycline GAR-936 at 0.12 μg/ml and evernimicin at 4 μg/ml, but it was resistant to linezolid. It was most susceptible to dirithromycin, quinupristin-dalfopristin, telithromycin, reference macrolides, and josamycin. *M. hominis* was susceptible to linezolid, evernimicin, and GAR-936. It was resistant to macrolides and the ketolide telithromycin but susceptible to quinupristin-dalfopristin and josamycin. *U. urealyticum* was susceptible to evernimicin (8 to 16 μg/ml) and resistant to linezolid. It was less susceptible to GAR-936 (4.0 μg/ml) than to tetracycline (0.5 μg/ml). Telithromycin and quinupristin-dalfopristin were the most active agents against ureaplasmas (0.06 μg/ml). The new quinolone gatifloxacin was active against *M. pneumoniae* and *M. hominis* at 0.12 to 0.25 μg/ml and active against ureaplasmas at 1.0 μg/ml. The MICs of macrolides were markedly affected by pH, with an 8- to 32-fold increase in the susceptibility of *M. pneumoniae* as the pH increased from 6.9 to 7.8. A similar increase in susceptibility with increasing pH was also observed with ureaplasmas. Tetracyclines showed a fourfold increase of activity as the pH decreased 1 U, whereas GAR-936 showed a fourfold decrease in activity with a decrease in pH.

The pattern of susceptibilities of mycoplasmas to antimicrobial agents is unique in that mycoplasmas do not have a cell wall that is the target for antibacterial agents like penicillin and the cephalosporins. Human mycoplasmas and ureaplasmas are generally susceptible to tetracyclines and quinolones (1, 9, 14, 17, 18). *Mycoplasma pneumoniae* and *Mycoplasma genitalium* are exquisitely sensitive to macrolides, whereas *Mycoplasma hominis* is naturally resistant (1, 6, 9, 10, 21, 32). Ureaplasmas are moderately susceptible to macrolides (9, 15, 21, 26, 32). Tetracycline resistance has been observed in both *M. hominis* and *Ureaplasma urealyticum* due to the *tetM* determinant (24, 25) but not yet in *M. pneumoniae*. Resistance to quinolones such as ofloxacin and sparfloxacin has been observed in clinical isolates of *M. hominis* (5). Resistance to erythromycin was observed long ago in *M. pneumoniae* (19, 31).

The increase in resistance of common pathogens to antimicrobial agents has prompted a search for new and improved antimicrobial agents (20). We report the comparative susceptibilities, as determined by the agar dilution method, for *M. pneumoniae*, *M. hominis*, and *U. urealyticum* to nine new antimicrobial agents: a glycylcycline (GAR-936), dalfopristin, dirithromycin, evernimicin (SCH27899), gatifloxacin, linezolid, moxifloxacin, quinupristin-dalfopristin, and telithromycin in comparison with reference macrolides, quinolones, and tetracyclines. We determined the effect of medium pH and report that pH has significant effects on the susceptibilities of mycoplasmas to macrolides and lesser effects on tetracyclines and glycylcyclines. A further purpose was to define the susceptibilities of mycoplasmas to antimicrobial agents as measured by the agar dilution method using controlled inocula and pHs.

(Some of these data have been presented in preliminary publications [G. E. Kenny and F. D. Cartwright, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother, p. 302, 1999; G. E. Kenny and F. D. Cartwright, Abstr. Int. Congr. Mycoplasmol., p. 197, 2000].)

MATERIALS AND METHODS

Mycoplasmas. The clinical isolates and prototypic strains of *M. pneumoniae*, *M. hominis*, and *U. urealyticum* were described previously (14, 17). An erythromycin-resistant strain of *M. pneumoniae* (PN 51) was an isolate from a pneumonia patient in 1963. New *M. pneumoniae* strains from the 1990s were kindly supplied by G. Cassell (University of Alabama, Birmingham) and Deborah Talkington (Centers for Disease Control and Prevention, Atlanta, Ga.). Strains were grown in H broth (13) supplemented with indicator metabolites: 5 mM glucose (*M. pneumoniae*) and 5 mM arginine (*M. hominis*). U broth (13) was used for *U. urealyticum*; it contains 5 mM urea and is buffered to pHs 6.3 to 6.5.

Antimicrobial agents. The macrolides tested were as follows: azithromycin (Charles, Pfizer, Groton, Conn.), clarithromycin (Abbott Laboratories, North Chicago, Ill.), dirithromycin (Eli Lilly, Indianapolis, Ind.), erythromycin (Sigma Chemical Co., St. Louis, Mo.), and roxithromycin (Aventis, Romainville, France). The ketolide telithromycin and the lincosamide josamycin were also supplied by Aventis. Macrolides and ketolides were solubilized in alcohol. The streptogramins dalfopristin and quinupristin-dalfopristin were obtained from Aventis and were solubilized in water. Linezolid (Pharmacia Upjohn, Kalamazoo, Mich.) was solubilized in water with a small amount of NaOH added. The everninomicin derivative evernimicin (SCH27899; Schering-Plough Research Institute, Ken-

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ilworth, N.J.) was solubilized in water. The quinolones tested were gatifloxacin (Bristol-Myers Squibb, Wallingford, Conn.), grepafloxacin (OPC 17116; Otsuka, New York, N.Y.), levofloxacin (Aventis), moxifloxacin (Bayer, West Haven, Conn.), sparfloxacin (Parke Davis, Ann Arbor, Mich.), and trovafloxacin (Pfizer, Groton, Conn.). Quinolones were solubilized in water aided by the addition of small amounts of NaOH. The glycylcycline (GAR-936; Wyeth-Ayerst, Pearl River, N.Y.) and the tetracyclines were soluble in water.

Susceptibility testing. Agar dilution testing using a Steers replicator was carried out as previously described (14, 17). Unless otherwise specified, the pH of the H agar medium for *M. hominis* and *M. pneumoniae* was 7.2 to 7.4 and that of the U agar medium (Kenny and Cartwright, Abstr. Int. Congr. Mycoplasmol.) for *U. urealyticum* was 6.3 to 6.5. Solutions of agents were adjusted for specific activity and prepared on the day that the agar plates were poured. The endpoint was that amount of agent that completely prevented formation of colonies on plates inoculated with 30 to 300 CFU per spot. These inoculum levels were achieved by plating three 10-fold dilutions of actively growing cultures with the dilutions being chosen as those most likely to yield 30 to 300 colonies on one of the replicator spots. The incubation times at 37°C in air were 4 days for ureaplasmas, 5 days for *M. hominis*, and 14 days for *M. pneumoniae*. These incubation times were twice the time periods needed for formation of microscopically visible colonies on the control plates.

Control of pH. Buffers with pK_as selected to span the pH range of 6.1 to 8.3 were prepared by dissolving 0.33 mol each of BICINE [*N*,*N*-bis(2-hydroxyethyl) glycine; pK_a 8.3 at 25°C], MES [2-(*N*-morpholino)ethanesulfonic acid; pK_a 6.1 at 25°C], and TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid: pK_a 7.4 at 25°C; Sigma Chemical Co.] in 500 ml of water at 37°C. Portions (100 ml) of this mixture were titrated with 10 N NaOH to give the individual pH values needed for the experiments. Then the volume was made up to 200 ml, resulting in concentrations of 0.33 M for each of the three buffer compounds, and the pH was tested again. The buffers were sterilized by filtration. Three milliliters of appropriately adjusted buffer was added to 97 ml of complete H agar medium (with 20% horse serum), resulting in a concentration for each buffer component of 10 mM. The pH was measured with a surface electrode on plates incubated in an air incubator for 14 days. Plates were incubated in sealed containers to prevent dehydration of plates and possible change in pH.

RESULTS

***M. hominis*.** Linezolid and evernimicin showed modest activity against *M. hominis* at MICs at which 50% of the isolates tested were inhibited (MIC_{50}s) of 4.0 to 8.0 μg/ml (Table 1). The glycylcycline GAR-936 was as active as minocycline against tetracycline-susceptible strains of *M. hominis*. Eight tetracycline-resistant strains of *M. hominis* (MICs of tetracycline and minocycline, >32) containing the TetM gene were as susceptible to GAR-936 (range, 0.12 to 0.25 μg/ml) as susceptible strains. *M. hominis* is known to be intrinsically resistant to macrolides. This was true in our study (Table 1). Slight susceptibilities were seen to azithromycin and telithromycin. It was susceptible to the lincosamide josamycin, quinupristin-dalfopristin, and dalfopristin. *M. hominis* was as susceptible to gatifloxacin (0.06 to 0.25 μg/ml) as to grepafloxacin. Sparfloxacin, moxifloxacin, and trovafloxacin were fourfold more active and levofloxacin and ciprofloxacin were fourfold less active than gatifloxacin. The tetracycline-resistant strains had the same susceptibility to quinolones as the tetracycline-susceptible strains.

***U. urealyticum*.** Evernimicin was active against *U. urealyticum* at 8 to 16 μg/ml (Table 2), but linezolid was not active at >64 μg/ml. Tetracycline-susceptible ureaplasmas were not as susceptible to GAR-936 (MIC_{50}, 4 μg/ml) as they were to tetracycline and minocycline (MIC_{50}s, 0.25 to 1.0 μg/ml). Three TetM gene-containing strains resistant to tetracycline and minocycline at >32 μg/ml were susceptible to GAR-936 at 8 to 16 μg/ml. Ureaplasmas have been considered to be susceptible to macrolides. The highest activity was shown by clarithromycin at 0.12 μg/ml (Table 2); the other macrolides were less active at 0.5 to 8.0 μg/ml (MIC_{90}s). The new agents quinupristin-dalfopristin and telithromycin were the most active antimicrobials against ureaplasmas at 0.06 μg/ml (MIC_{50}). Ureaplasmas were susceptible to quinolones, with the highest activities being shown by moxifloxacin and sparfloxacin. Gatifloxacin had the same activity as ofloxacin and levofloxacin.

TABLE 1. Susceptibilities of *M. hominis* to new antimicrobial agents[a] compared with those to older reference agents

Agent (no. of strains)	MIC (μg/ml)		
	Range	50%	90%
New agents			
Evernimicin (43)	**1.0–16.0**	**4.0**	**4.0**
Linezolid (24)	**2.0–8.0**	**8.0**	**8.0**
Tetracyclines, glycylcycline			
GAR-936 (29)[b]	**0.125–0.5**	**0.25**	**0.5**
Tetracycline (29)[b]	0.5–4.0	1.0	2.0
Minocycline (29)[b]	0.06–0.5	0.125	0.125
GAR-936 (8)[c]	**0.125–0.5**	**0.25**	
Tetracycline (8)[c]	>32	>32	
Minocycline (8)[c]	>32	>32	
Macrolides, lincosamide, streptogramins, ketolide			
Dirithromycin (12)	**>32**	**>32**	**>32**
Dalfopristin (29)	**0.5–2.0**	**1.0**	**2.0**
Quinupristin-dalfopristin (29)	**0.03–0.125**	**0.06**	**0.06**
Telithromycin (43)	**16–32**	**32**	**32**
Azithromycin (43)	16–32	32	32
Clarithromycin (12)	>32	>32	>32
Erythromycin (34)	>32	>32	>32
Roxithromycin (12)	>32	>32	>32
Josamycin (12)	0.25–0.5	0.25	0.25
Quinolones			
Gatifloxacin (43)	**0.06–0.25**	**0.12**	**0.12**
Moxifloxacin (35)	**0.015–0.06**	**0.03**	**0.06**
Ciprofloxacin (43)	0.25–1.0	0.5	2.0
Grepafloxacin (23)	0.06–0.125	0.12	0.12
Levofloxacin (32)	0.25–2.0	0.25	0.5
Sparfloxacin (41)	0.015–0.03	0.03	0.03
Trovafloxacin (43)	0.015–0.125	0.03	0.06

[a] Bold type indicates new agents.
[b] Tetracycline-susceptible strains.
[c] Tetracycline-resistant strains.

***M. pneumoniae*.** *M. pneumoniae* was resistant to linezolid (Table 3) and susceptible to evernimicin (2.0 to 4.0 μg/ml). The new glycylcycline GAR-936 was fourfold more active than tetracycline or minocycline. The macrolides were highly active against *M. pneumoniae* (Table 3), with MIC_{50}s ranging from 0.015 μg/ml (azithromycin) to 0.25 μg/ml (dirithromycin). The streptogramin quinupristin-dalfopristin and the ketolide telithromycin were also active (MIC_{50}s, 0.015 to 0.03 μg/ml). An erythromycin-resistant strain was resistant to azithromycin, clarithromycin, and dirithromycin but susceptible to quinupristin-dalfopristin. This strain was as susceptible to tetracyclines and quinolones as the erythromycin-susceptible strains. The new quinolones moxifloxacin and gatifloxacin were as active as grepafloxacin, moxifloxacin, sparfloxacin, and trovafloxacin (0.12 to 0.25 μg/ml).

Effects of pH. The pH had a large effect on the MICs of selected macrolides for *M. pneumoniae* (Table 4). The four

TABLE 2. Susceptibilities of *U. urealyticum* to new antimicrobial agents[a] compared with those to older agents for reference

Agent (no. of strains)	MIC (μg/ml)		
	Range	50%	90%
New agents			
Evernimicin (22)	**8.0–16.0**	**16.0**	**16.0**
Linezolid (11)	**>64**	**>64**	**>64**
Tetracyclines, glycylcycline			
GAR-936 (25)	**1.0–16.0**	**4.0**	**8.0**
Tetracycline (25)	0.5–4.0	0.5	1.0
Minocycline (25)	0.06–0.5	0.25	0.25
Macrolides, lincosamide, streptogramins, ketolide			
Dirithromycin (33)	**1.0–4.0**	**1.0**	**2.0**
Dalfopristin (34)	**0.5–4.0**	**2.0**	**2.0**
Quinupristin-dalfopristin (34)	**0.03–0.25**	**0.06**	**0.12**
Telithromycin (33)	**0.06–0.25**	**0.06**	**0.25**
Azithromycin (33)	0.25–0.5	0.5	0.5
Clarithromycin (33)	≤0.06–2.0	0.12	0.12
Erythromycin (33)	1.0–8.0	2.0	8.0
Roxithromycin (33)	1.0–4.0	2.0	4.0
Josamycin (21)	0.5–2.0	0.5	2.0
Quinolones			
Gatifloxacin (34)	**1.0–2.0**	**1.0**	**1.0**
Levofloxacin (8)	1.0–2.0	1.0	2.0
Moxifloxacin (34)	**0.25–1.0**	**0.25**	**0.5**
Ofloxacin (34)	1.0–2.0	2.0	2.0
Sparfloxacin (34)	0.25–0.5	0.25	0.5

[a] Bold type indicates new agents.

macrolides were much less active at pH 6.9 than at pH 7.8: 32-fold less active for azithromycin and dirithromycin and 8-fold less active for clarithromycin and erythromycin (Table 4). If we consider MICs at pHs 7.2 and 7.5 as being the most clinically relevant values, then these macrolides were highly active, with MIC_{50}s ranging from 0.004 to 0.25 μg/ml. The relative order of MIC_{50}s in this pH range was as follows: 0.004 to 0.015 μg/ml for azithromycin, 0.015 to 0.03 μg/ml for clarithromycin, 0.015 to 0.06 μg/ml for erythromycin, and 0.03 to 0.25 μg/ml for dirithromycin. Telithromycin was only twofold less active at pH 6.9 than at pH 7.3. Ureaplasmas also showed a pH effect, with susceptibility toward macrolides increasing at higher pH values. These macrolides were least active at pH 6.0 and showed four- to eightfold greater activity at pH 6.7 (the highest pH at which ureaplasmas form recognizable colonies). The susceptibilities were as follows: erythromycin, 8 μg/ml at pH 6.0 and 1.0 μg/ml at pH 6.7; azithromycin, 1.0 μg/ml at pH 6.0 and 0.25 μg/ml at pH 6.7; clarithromycin, 0.5 μg/ml at pH 6.0 and 0.0625 μg/ml at pH 6.7; dirithromycin, 1.0 μg/ml at pH 6.0 and 0.25 μg/ml at pH 6.7. The susceptibility of *M. hominis* to josamycin was affected eightfold by pH: the MIC_{50}s of 10 strains were 0.25 μg/ml at pH 7.6 and 2.0 μg/ml at pH 6.6. Thirteen strains of *M. hominis* were fourfold less susceptible to GAR-936 at pH 6.4 (1.0 μg/ml) than at pH 7.4 (0.25 μg/ml). In contrast, *M. hominis* was fourfold more susceptible to tetracycline at pH 6.4 (0.25 μg/ml) than at pH 7.4 (1.0 μg/ml). When susceptibilities to ciprofloxacin, gatifloxacin, levofloxacin, ofloxacin, and trovafloxacin were tested with *M. hominis* at pHs 6.4 and 7.4, no changes greater than twofold were observed. *M. hominis* showed no change in susceptibility to quinupristin-dalfopristin when it was tested at pHs 6.6 to 7.6.

DISCUSSION

Two new classes of antimicrobial agents were evaluated. Linezolid, a member of the oxazolidinone group, is a multicyclic compound that is active against gram-positive bacteria apparently by inhibiting protein synthesis upon binding to the 50S ribosomal subunit (29). Linezolid is active against *M. hominis* (MIC_{50}, 8.0 μg/ml) but not against *U. urealyticum* or *M. pneumoniae*. The everninomicin group of oligosaccharide antibiotics represented by evernimicin (SCH27899) blocks formation of the 50S ribosomal subunit and inhibits translation (7). Both *M. pneumoniae* and *M. hominis* are susceptible at 4 μg/ml (MIC_{50}), whereas ureaplasmas are susceptible only at 16 μg of evernimicin per ml. Evernimicin is active against gram-positive bacteria, with MIC_{50}s of 0.12 to 1.0 μg/ml (7).

The activity of the new glycylcycline GAR-936 is similar to those shown by the *N*,*N*-dimethylglycyl amido derivatives of minocycline and 6-demethyl-6-deoxytetracycline reported previously (16). Both *M. pneumoniae* (MIC_{50}, 0.12 μg/ml) and *M. hominis* (0.25 μg/ml) are fourfold-more susceptible to GAR-936 than to tetracycline (Tables 1 and 3). Strains of *M. hominis* containing the TetM gene are as susceptible to GAR-936 as tetracycline-susceptible strains. Ureaplasmas are susceptible to GAR-936 at an MIC_{50} of 4 μg/ml. As with the previous glycylcyclines (16), GAR-936 became less active with decreasing pH whereas minocycline and tetracycline became more active as pH decreased. The acid pH required for growth of ureaplasmas likely explains their poor susceptibilities to GAR-936.

The macrolide, lincosamide, streptogramin, and ketolide

TABLE 3. Susceptibilities of *M. pneumoniae* to new antimicrobial agents[a] compared with those to older reference agents

Agent (no. of strains)	MIC (μg/ml)		
	Range	50%	90%
New agents			
Linezolid (24)	**>64**	**>64**	**>64**
Evernimicin (40)	**2.0–4.0**	**4.0**	**4.0**
Tetracyclines, glycylcycline (GAR-936)			
GAR-936 (30)	**0.06–0.25**	**0.12**	**0.25**
Tetracycline (30)	0.5–2.0	0.5	1.0
Minocycline (30)	0.25–1.0	0.5	1.0
Macrolides, lincosamide, streptogramins, ketolide			
Dirithromycin (45)	**0.12–0.5**	**0.25**	**0.25**
Dalfopristin (48)	**0.06–1.0**	**0.25**	**0.5**
Quinupristin-dalfopristin (48)	**0.004–0.06**	**0.015**	**0.03**
Telithromycin (47)	**0.008–0.06**	**0.008**	**0.008**
Azithromycin (45)	0.008–0.12	0.015	0.03
Clarithromycin (45)	0.015–0.06	0.03	0.03
Erythromycin (45)	0.03–0.12	0.06	0.06
Roxithromycin (40)	0.06–0.25	0.12	0.25
Josamycin (21)	0.03–0.12	0.03	0.06
Quinolones			
Gatifloxacin (41)	**0.25–1.0**	**0.25**	**0.5**
Grepafloxacin (41)	0.06–0.25	0.12	0.25
Levofloxacin (41)	0.5–2.0	1.0	2.0
Moxifloxacin (35)	**0.12**	**0.12**	**0.12**
Sparfloxacin (40)	0.12–0.25	0.25	0.25
Trovafloxacin (40)	0.12–0.5	0.25	0.5

[a] Bold type indicates new agents.

TABLE 4. Effect of pH on the susceptibilities of *M. pneumoniae* to macrolides

pH (no. of strains tested)	MIC_{50} (μg/ml) (range)			
	Azithromycin	Clarithromycin	Dirithromycin	Erythromycin
6.9 (45)	0.03 (0.05–0.06)	0.03 (0.015–0.03)	0.25 (0.25–0.5)	0.25 (0.12–0.25)
7.1 (45)	0.015 (0.007–0.015)	0.03 (0.015–0.06)	0.25 (0.12–0.5)	0.06 (0.03–0.12)
7.5 (22)	0.002 (0.002–0.007)	0.015 (0.007–0.015)	0.03 (0.015–0.12)	0.015 (0.007–0.03)
7.8 (45)	0.001 (0.0005–0.001)	0.004 (0.002–0.007)	0.007 (≤0.004–0.007)	0.015 (≤0.015–0.03)

groups of antimicrobial agents had high activities against *M. pneumoniae*. The MIC_{50}s of four macrolides for *M. pneumoniae* ranged from 0.015 to 0.25 μg/ml (Table 4) at pHs 7.1 to 7.5. The MIC_{50}s reported in the literature have been as much as 50-fold lower, with values as small as 0.00024 to 0.0039 μg/ml (1, 4, 9–12, 14, 18, 21, 22, 26, 28, 30–32). One reason for this difference is that the medium for mycoplasmas is usually adjusted to pH 7.6 or greater (8). The apparent in vitro activities of erythromycin and azithromycin against bacteria are known to increase as the pH of the medium increases (23, 27). The mycoplasmal susceptibility method has additional problems in that 10 to 20% of animal serum is incorporated in the mycoplasmal medium. Serum contains various amounts of bicarbonate and will equilibrate to a higher pH at 37°C (23) unless the medium is buffered. We used buffered media and measured the pH after equilibration in the incubator. Some of the differences between our data and those in the literature lie in our finding that medium pH has an 8- to 32-fold effect on the apparent susceptibility of *M. pneumoniae* to macrolides when the pH is increased from 6.9 to 7.8 (Table 4). The largest pH effects were seen with azithromycin and dirithromycin. *M. pneumoniae* was susceptible to the ketolide telithromycin at the MIC_{50} of 0.008 μg/ml. Yamaguchi et al. (33) give a value of 0.00097 μg/ml. The streptogramin quinupristin-dalfopristin had an MIC_{50} of 0.03 μg/ml compared to reported values of 0.1 μg/ml (2) and 0.062 μg/ml (11). We did not find a pH effect with streptogramins when they were tested at pHs 6.6 to 7.6 with *M. hominis*.

U. urealyticum was most susceptible to clarithromycin at 0.12 μg/ml and susceptible to the other macrolides at 0.5 to 2.0 μg/ml (Table 2). The greater activity of clarithromycin might result from the lesser effect of pH on its activity (Table 4). Ureaplasmas were susceptible to both telithromycin and dalfopristin-quinupristin at 0.06 μg/ml. *M. hominis* showed slight susceptibility to azithromycin and telithromycin (Table 1) and was resistant to other macrolides. It was susceptible to the lincosamide josamycin and more susceptible to quinupristin-dalfopristin.

M. pneumoniae was as susceptible to the new quinolone gatifloxacin as it was to grepafloxacin, moxifloxacin, sparfloxacin, and trovafloxacin at 0.12 to 0.25 μg/ml. Against *M. hominis*, gatifloxacin at an MIC_{50} of 0.12 μg/ml was fourfold more active than ciprofloxacin and twofold more active than levofloxacin but fourfold less active than sparfloxacin and trovafloxacin. Against ureaplasmas, gatifloxacin was as active as levofloxacin and fourfold less active than either moxifloxacin or sparfloxacin.

Determining the susceptibilities of mycoplasmas and ureaplasmas to antimicrobial agents is particularly difficult (3) because cultures do not show turbidity and the inoculum cannot be standardized before the tests are run. By employing three inoculum dilutions, we were able to define susceptibilities in a range of 30 to 300 organisms per spot. Other studies have defined the inoculum level by employing a tube dilution method using one tube per dilution to determine the inoculum. Such a system gives a 10-fold or greater variation in the inocula. Our previous study (15) showed a large effect of inoculum size on MIC. The reproducibility of a method also can be judged from the range of the data for a given agent with susceptible wild-type strains. The most common high-low range in our study reflected a fourfold difference (Tables 1 to 3). Standardization of medium pH and inoculum size appears necessary for obtaining reliable MICs for mycoplasmas and ureaplasmas.

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